Map of the Human type IX collagen molecule showing the 3α chains which constitute the heteropolymer and their collagenous (COL) and non-collagenous (NC) domains.

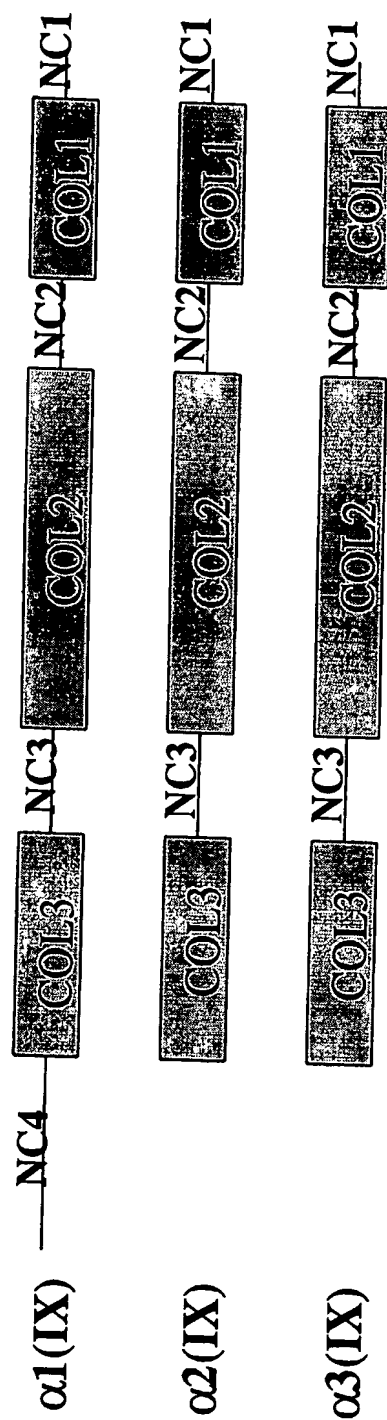

Figure 1

Figure 2

Schema for separation and fractionation of Peptacans into GAG-peptides and polypeptides

Bovine tracheal cartilage powder

↓

Autolysis at pH 4.5 Na⁺ or Ca⁺⁺ buffers

↓

| **Residue (freeze)** | **Supernatant** |

↓

Ion exchange media

↓

| **Bound GAG-complexes** | **Unboundpeptides in washes** |

| **Re-cycle if required** | **Buffer washes** | **Diafiltrate** |

↓

| **Release buffer** | **chromatography** |

↓

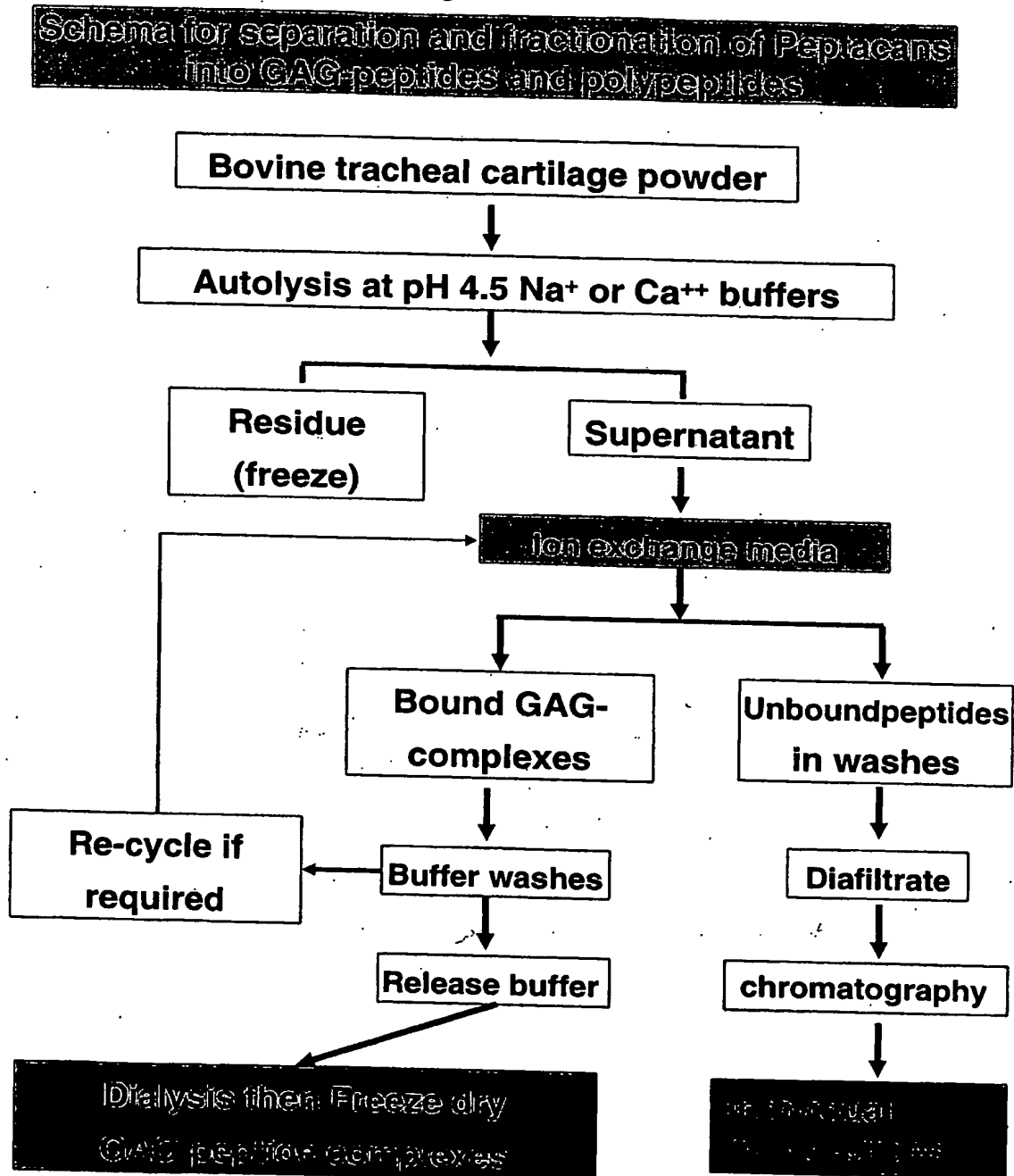Dialysis then Freeze dry GAG peptide complexes

Figure 3

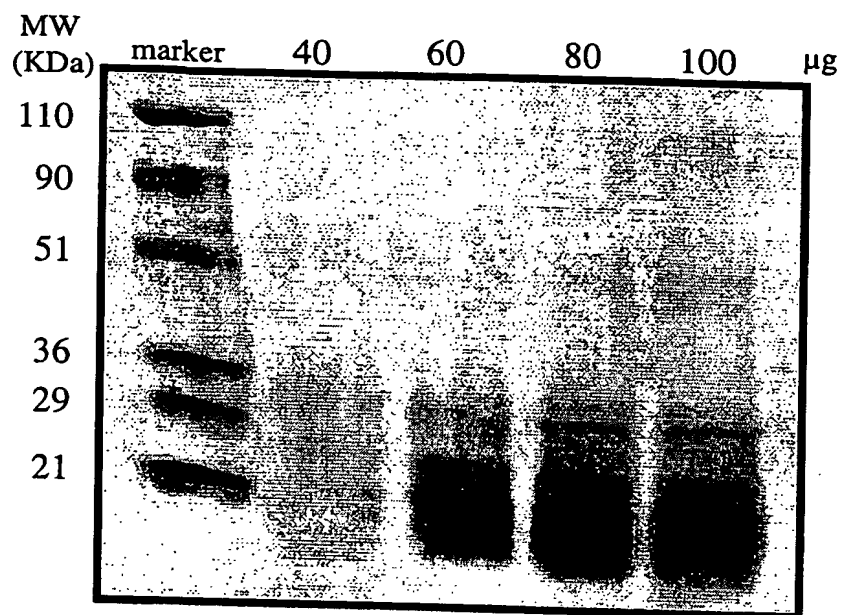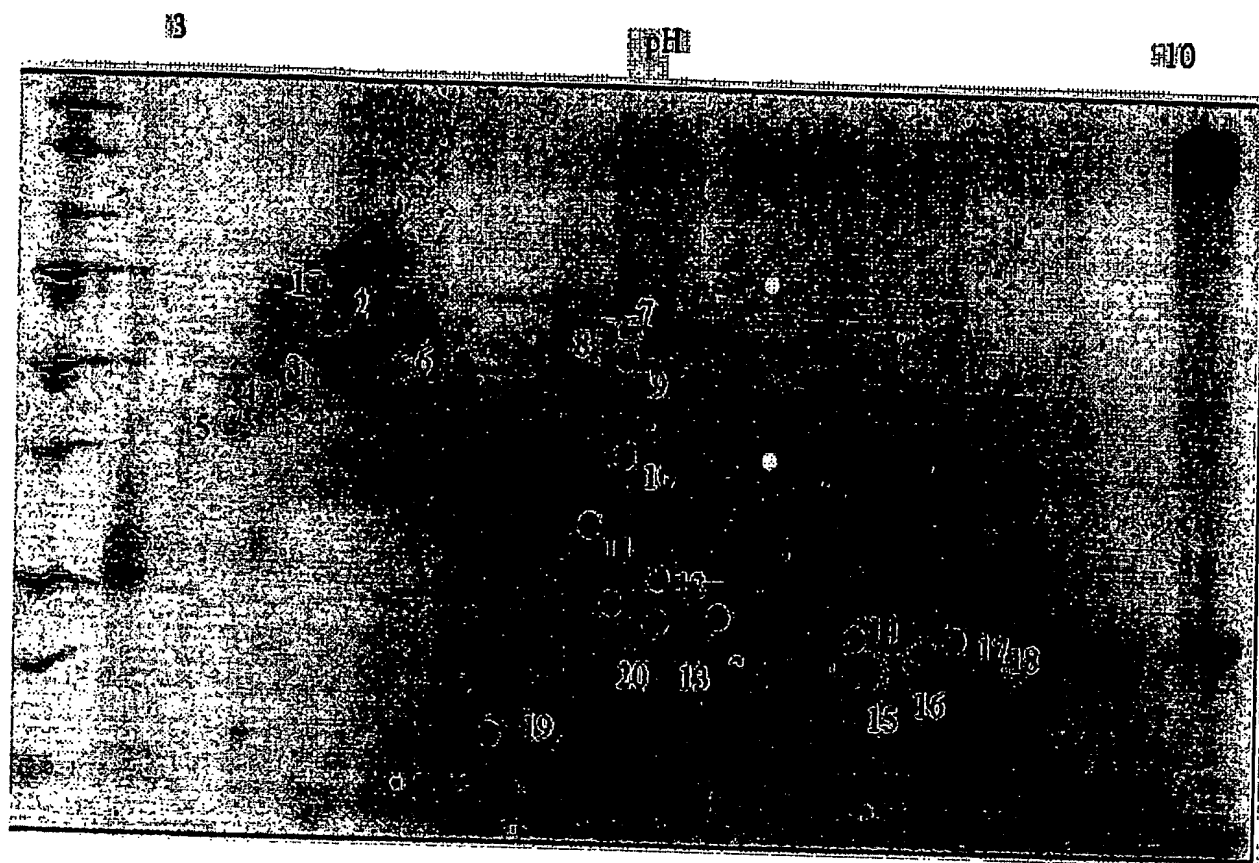## SDS-PAGE of Proteins (polypeptides) Isolated from CaP

Figure 4

CBT protein sample, pH 3-10 gradient. Proteins annotated for MALDI-MS.

FIGURE 5

1

Tentative

5 NCF1_BOVIN

Neutrophil cytosol factor 1

Molecular weight: 45346

Matches: 5

MOWSE Score: 1.6076937e+003

10 Likelihood: 1.96e+003

Coverage: 14.80 %

Matching peptides:

MW Delta Start End Sequence

841.4657 -88.56 120 126 (K)VRPDD LK(L)

15 886.4760 -21.99 127 134 (K)LPTDS QVK(K)

886.4508 -50.37 283 291 (K)AGQDV AQAK(S)

* 1164.5822 0.02 328 336 (R)NSVRF MQQR(R)

1730.7934 -0.01 56 70 (K)EMFPI EAGDI NPENR(I)

1891.9197 23.26 170 188 (K)GSSSQ MALAT GDVVD VVEK(N)

20 **OR**

Tentative

ALBU_BOVIN

Bovine Serum Albumin

Molecular weight: 69294

25 Matches: 5

MOWSE Score: 7.6716406e+001

Likelihood: 1.88e+003

Coverage: 8.73 %

Matching peptides:

30 MW Delta Start End Sequence

711.3664 59.75 29 34 (K)SEIAH R(F)

* 959.5400 -35.02 210 218 (R)EKVLA SSAR(Q)

* 1000.5818 -23.99 233 241 (R)ALKAW SVAR(L)

1385.6133 -28.71 286 297 (K)YICDN QDTIS SK(L)

35 * 1961.9404 26.67 139 155 (K)LKPDP NTLCD EFKAD EK(K)

2

Tentative

Score: 0.19, 6 matching peptides: P35445 (COMP_BOVIN) pI: undefined, Mw: undefined

Cartilage oligomeric matrix protein (COMP) (Fragment). - Bos taurus
5 **(Bovine).**

user mass matching [Delta] #MC modification positionpeptide

mass (ppm) mass

887.4908 887.4404 -56.88 0 Cys_PAM: 10 8-14 DNCPLVR

1181.5063 1181.4463 -50.84 0 2xCys_PAM 26-34 WGDACDNCR

10 1226.6927 1226.631 -50.35 1 Cys_PAM: 69 62-71 IRNPVDNCPK

1337.5306 1337.491 -29.64 0 Cys_PAM: 53 50-61 GDACDDDIDGDR

Page 3

1370.707 1370.6369 -51.19 0 168-179 LVPNPGQEDMDR

1386.6544 1386.6318 -16.32 0 MSO: 177 168-179 LVPNPGQEDMDR

15 11.4% of sequence covered:

3

No Good Match

4

No Good Match

20 **5**

No Good Match

6

No Good Match

7

25 ALBU_BOVIN

Bovine Serum Albumin

Molecular weight: 69294

Matches: 11

MOWSE Score: 9.5664269e+006

30 Likelihood: 4.27e+003

Coverage: 17.96 %

Matching peptides:

MW Delta Start End Sequence

926.4862 -137.94 161 167 (K)YLYEI AR(R)

35 1162.6234 -74.58 66 75 (K)LVNEL TEFAK(T)

1282.7033 -96.76 361 371 (R)HPEYA VSVLL R(L)

1304.7088 -90.40 402 412 (K)HLVDE PQNLI K(Q)

* 1438.8045 -82.02 360 371 (R)RHPEY AVSVL LR(L)

1478.7881 -92.19 421 433 (K)LGEYG FQNAL IVR(Y)

1510.8355 -83.71 438 451 (K)VPQVS TPTLV EVSR(S)

5   1566.7354 -87.40 347 359 (K)DAFLG SFLYE YSR(R)

* 1638.9304 -66.09 437 451 (R)KVPQV STPTL VEVSR(S)

1414.6802 -38.28 569 580 (K)TVMEN FVAFV DK(C)

+ Methionine Sulfoxide

1893.9294 -61.74 508 523 (R)RPCFS ALTPD ETYVP K(A)

10 + Cysteine acrylamide

Page 4

8

ALBU_BOVIN

Bovine Serum Albumin

15 **Molecular weight: 69294**

Matches: 13

MOWSE Score: 7.6034479e+007

Likelihood: 1.17e+004

Coverage: 21.42 %

20 Matching peptides:

MW Delta Start End Sequence

926.4862 -72.22 161 167 (K)YLYEI AR(R)

1162.6234 -28.39 66 75 (K)LVNEL TEFAK(T)

1282.7033 -47.50 361 371 (R)HPEYA VSVLL R(L)

25 1304.7088 -31.77 402 412 (K)HLVDE PQNLI K(Q)

* 1438.8045 -39.49 360 371 (R)RHPEY AVSVL LR(L)

1478.7881 -32.08 421 433 (K)LGEYG FQNAL IVR(Y)

1510.8355 -19.52 438 451 (K)VPQVS TPTLV EVSR(S)

1518.7388 -25.79 139 151 (K)LKPDP NTLCD EFK(A)

30 1566.7354 -34.55 347 359 (K)DAFLG SFLYE YSR(R)

* 1638.9304 -19.36 437 451 (R)KVPQV STPTL VEVSR(S)

* 988.5488 24.47 221 228 (R)LRCAS IQK(F)

+ Cysteine acrylamide

1414.6802 8.58 569 580 (K)TVMEN FVAFV DK(C)

35 + Methionine Sulfoxide

1893.9294 -10.63 508 523 (R)RPCFS ALTPD ETYVP K(A)

+ Cysteine acrylamide

9

NCF1_BOVIN

10

5   Q95L50

Type IX collagen alpha 1 chain
Molecular weight: 20907
Matches: 5
MOWSE Score: 3.7725965e+003
10 Likelihood: 5.46e+003
Coverage: 28.34 %
Matching peptides:
MW Delta Start End Sequence
933.4668 -63.69 61 68 (K)LGNNV DFR(I)
15 1051.6390 -43.95 173 181 (R)IESLP IKPR(G)
2183.0687 42.58 73 90 (R)HLYPN GLPEE YSFLT TFR(M)
832.4476 -30.07 155 161 (K)IMIGV ER(S)
+ Methionine Sulfoxide
1282.5976 -22.54 162 172 (R)SSATL FVDCN R(I)
20 + Cysteine acrylamide

11

ALBU_BOVIN

Bovine Serum Albumin
25 **Molecular weight: 69294**
Matches: 5
MOWSE Score: 1.1175735e+003
Likelihood: 7.26e+002
Coverage: 10.21 %
30 Matching peptides:
MW Delta Start End Sequence
926.4862 -156.39 161 167 (K)YLYEI AR(R)
1282.7033 -36.27 361 371 (R)HPEYA VSVLL R(L)
1566.7354 -84.65 347 359 (K)DAFLG SFLYE YSR(R)
35 * 1887.9876 -6.32 89 105 (K)SLHTL FGDEL CKVAS LR(E)
1887.9195 -42.39 169 183 (R)HPYFY APELL YYANK(Y)

1790.7021 -70.93 267 280 (K)ECCHG DLLEC ADDR(A)
+ Cysteine acrylamide
+ Cysteine acrylamide
+ Cysteine acrylamide

5 **12**
ALBU_BOVIN
Bovine Serum Albumin
Molecular weight: 69294
Matches: 16
10 MOWSE Score: 3.5119435e+010
Likelihood: 1.04e+004
Coverage: 28.17 %
Matching peptides:
MW Delta Start End Sequence
15 926.4862 -106.87 161 167 (K)YLYEI AR(R)
. 1282.7033 28.51 361 371 (R)HPEYA VSVLL R(L)
1304.7088 -33.76 402 412 (K)HLVDE PQNLI K(Q)
1478.7881 -58.18 421 433 (K)LGEYG FQNAL IVR(Y)
1510.8355 -34.08 438 451 (K)VPQVS TPTLV EVSR(S)
20 * 1638.9304 -30.89 437 451 (R)KVPQV STPTL VEVSR(S)
* 1737.8032 -39.00 387 401 (K)DDPHA CYSTV FDKLK(H)
1120.5223 -76.32 588 597 (K)EACFA VEGPK(L)
+ Cysteine acrylamide
1165.5220 -96.70 499 507 (K)CCTES LVNR(R)
25 + Cysteine acrylamide
+ Cysteine acrylamide
1193.5169 -102.89 460 468 (R)CCTKP ESER(M)
+ Cysteine acrylamide
+ Cysteine acrylamide
30 1414.6802 16.07 569 580 (K)TVMEN FVAFV DK(C)
+ Methionine Sulfoxide
1567.6613 9.82 387 399 (K)DDPHA CYSTV FDK(L)
+ Cysteine acrylamide
1589.7759 24.36 139 151 (K)LKPDP NTLCD EFK(A)
35 + Cysteine acrylamide
Page 6

Page 6

1753.8379 17.95 469 482 (R)MPCTE DYLSL ILNR(L)

+ Methionine Sulfoxide

+ Cysteine acrylamide

5   1893.9294 -3.13 508 523 (R)RPCFS ALTPD ETYVP K(A)

+ Cysteine acrylamide

1920.9291 10.74 529 544 (K)LFTFH ADICT LPDTE K(Q)

+ Cysteine acrylamide

13

10   Q95L50

Type IX collagen alpha 1 chain

Molecular weight: 20907

Matches: 9

MOWSE Score: 1.6928014e+007

15   Likelihood: 1.54e+004

Coverage: 62.03 %

Matching peptides:

MW Delta Start End Sequence

816.4018 -95.42 124 130 (K)SVSFS YK(G)

20   816.4527 -32.98 155 161 (K)IMIGV ER(S)

933.4668 -51.91 61 68 (K)LGNNV DFR(I)

1051.6390 -49.75 173 181 (R)IESLP IKPR(G)

1570.7528 -9.01 99 111 (K)HWSIW QIQDS SGK(E)

2148.0739 -6.99 21 39 (R)IGQDD LPGFD LISQF QIDK(A)

25   2183.0687 -12.48 73 90 (R)HLYPN GLPEE YSFLT TFR(M)

2618.2765 -0.04 131 154 (K)GLDGS LQTAA FSNLP SLFDS QWHK(I)

832.4476 -3.52 155 161 (K)IMIGV ER(S)

+ Methionine Sulfoxide

1282.5976 -44.29 162 172 (R)SSATL FVDCN R(I)

30   + Cysteine acrylamide

14

Q95L50

Type IX collagen alpha 1 chain

Molecular weight: 20907

35   Matches: 9

MOWSE Score: 1.6928014e+00

Likelihood: 8.48e+003

Coverage: 62.03 %

Matching peptides:

MW Delta Start End Sequence

5   816.4018 -156.04 124 130 (K)SVSFS YK(G)

816.4527 -93.60 155 161 (K)IMIGV ER(S)

933.4668 -138.67 61 68 (K)LGNNV DFR(I)

1051.6390 -126.57 173 181 (R)IESLP IKPR(G)

1570.7528 -78.20 99 111 (K)HWSIW QIQDS SGK(E)

10   2148.0739 -53.45 21 39 (R)IGQDD LPGFD LISQF QIDK(A)

Page 7

2183.0687 -61.45 73 90 (R)HLYPN GLPEE YSFLT TFR(M)

2618.2765 -0.10 131 154 (K)GLDGS LQTAA FSNLP SLFDS QWHK(I)

832.4476 -105.98 155 161 (K)IMIGV ER(S)

15   + Methionine Sulfoxide

1282.5976 -119.59 162 172 (R)SSATL FVDCN R(I)

+ Cysteine acrylamide

15

Q95L50

20   **Type IX collagen alpha 1 chain**

Molecular weight: 20907

Matches: 7

MOWSE Score: 5.0424749e+005

Likelihood: 8.67e+003

25   Coverage: 48.13 %

Matching peptides:

MW Delta Start End Sequence

816.4018 103.26 124 130 (K)SVSFS YK(G)

816.4527 165.71 155 161 (K)IMIGV ER(S)

30   933.4668 13.11 61 68 (K)LGNNV DFR(I)

1051.6390 5.50 173 181 (R)IESLP IKPR(G)

1570.7528 -0.05 99 111 (K)HWSIW QIQDS SGK(E)

2183.0687 -17.84 73 90 (R)HLYPN GLPEE YSFLT TFR(M)

2618.2765 0.11 131 154 (K)GLDGS LQTAA FSNLP SLFDS QWHK(I)

35   1282.5976 -29.95 162 172 (R)SSATL FVDCN R(I)

+ Cysteine acrylamide

16

Q95L50

Type IX collagen alpha 1 chain
Molecular weight: 20907

5 Matches: 5

MOWSE Score: 1.6468835e+004

Likelihood: 8.25e+003

Coverage: 34.76 %

Matching peptides:

10 MW Delta Start End Sequence

933.4668 35.82 61 68 (K)LGNNV DFR(I)

1051.6390 30.60 173 181 (R)IESLP IKPR(G)

2148.0739 0.08 21 39 (R)IGQDD LPGFD LISQF QIDK(A)

2183.0687 -1.95 73 90 (R)HLYPN GLPEE YSFLT TFR(M)

15 1282.5976 -7.80 162 172 (R)SSATL FVDCN R(I)

+ Cysteine acrylamide

Page 8

17

Q95L50

20 **Type IX collagen alpha 1 chain**
Molecular weight: 20907

Matches: 8

MOWSE Score: 9.0085540e+005

Likelihood: 5.86e+003

25 Coverage: 49.20 %

Matching peptides:

MW Delta Start End Sequence

816.4018 -137.06 124 130 (K)SVSFS YK(G)

816.4527 -74.62 155 161 (K)IMIGV ER(S)

30 933.4668 -125.07 61 68 (K)LGNNV DFR(I)

1051.6390 -117.92 173 181 (R)IESLP IKPR(G)

1570.7528 -50.26 99 111 (K)HWSIW QIQDS SGK(E)

2148.0739 -4.71 21 39 (R)IGQDD LPGFD LISQF QIDK(A)

2183.0687 -24.99 73 90 (R)HLYPN GLPEE YSFLT TFR(M)

35 832.4476 -86.04 155 161 (K)IMIGV ER(S)

+ Methionine Sulfoxide

1282.5976 -97.22 162 172 (R)SSATL FVDCN R(I)

+ Cysteine acrylamide

18

Q95L50

5 **Type IX collagen alpha 1 chain**

Molecular weight: 20907

Matches: 5

MOWSE Score: 1.6468835e+004

Likelihood: 2.56e+003

10 Coverage: 34.76 %

Matching peptides:

MW Delta Start End Sequence

933.4668 -122.28 61 68 (K)LGNNV DFR(I)

1051.6390 -107.17 173 181 (R)IESLP IKPR(G)

15 2148.0739 0.02 21 39 (R)IGQDD LPGFD LISQF QIDK(A)

2183.0687 -6.21 73 90 (R)HLYPN GLPEE YSFLT TFR(M)

1282.5976 -90.21 162 172 (R)SSATL FVDCN R(I)

+ Cysteine acrylamide

19

20 OBP_BOVIN

Odorant-binding protein

Molecular weight: 18503

Matches: 7

MOWSE Score: 4.8550116e+005

25 Likelihood: 2.71e+004

Coverage: 50.94 %

Page 9

Matching peptides:

MW Delta Start End Sequence

30 959.4825 -4.49 30 37 (K)IQENG PFR(T)

993.4655 32.50 42 49 (R)ELVFD DEK(G)

1161.5706 1.66 50 59 (K)GTVDF YFSVK(R)

1207.6085 18.62 19 29 (R)TVYIG STNPE K(I)

1359.7259 -11.18 97 108 (R)THLVA HNINV DK(H)

35 1788.8067 1.86 145 159 (K)NVVNF LENED HPHPE(-)

1947.8486 2.44 74 90 (K)QDDGT YVADY EGQNV FK(I)

20

Q95L50

Type IX collagen alpha 1 chain
Molecular weight: 20907

5 Matches: 8

MOWSE Score: 9.0085540e+005

Likelihood: 6.84e+003

Coverage: 49.20 %

Matching peptides:

10 MW Delta Start End Sequence

816.4018 -121.38 124 130 (K)SVSFS YK(G)

816.4527 -58.95 155 161 (K)IMIGV ER(S)

933.4668 -106.00 61 68 (K)LGNNV DFR(I)

1051.6390 -108.98 173 181 (R)IESLP IKPR(G)

15 1570.7528 -54.97 99 111 (K)HWSIW QIQDS SGK(E)

2148.0739 -18.35 21 39 (R)IGQDD LPGFD LISQF QIDK(A)

2183.0687 -25.77 73 90 (R)HLYPN GLPEE YSFLT TFR(M)

832.4476 -81.84 155 161 (K)IMIGV ER(S)

+ Methionine Sulfoxide

20 1282.5976 -93.79 162 172 (R)SSATL FVDCN R(I)

+ Cysteine acrylamide

21

Q95L50

Type IX collagen alpha 1 chain

25 **Molecular weight: 20907**

Matches: 8

MOWSE Score: 9.0085540e+005

Likelihood: 6.26e+003

Coverage: 49.20 %

30 Matching peptides:

MW Delta Start End Sequence

816.4018 -133.87 124 130 (K)SVSFS YK(G)

816.4527 -71.44 155 161 (K)IMIGV ER(S)

933.4668 -113.93 61 68 (K)LGNNV DFR(I)

35 1051.6390 -115.35 173 181 (R)IESLP IKPR(G)

1570.7528 -60.83 99 111 (K)HWSIW QIQDS SGK(E)

2148.0739 -9.04 21 39 (R)IGQDD LPGFD LISQF QIDK(A)
2183.0687 -23.66 73 90 (R)HLYPN GLPEE YSFLT TFR(M)
Page 10
832.4476 -68.62 155 161 (K)IMIGV ER(S)

5   + Methionine Sulfoxide

1282.5976 -93.25 162 172 (R)SSATL FVDCN R(I)
+ Cysteine acrylamide

FIGURE 6

Physical Characteristics of Protein fragments found in Calcium Peptacan after ion-exchange treatment and 2D electrophoresis.

Fragment ID#	Estimated isoelectric point	Estimated Molecular Weight
1	4.0	74kDa
2	4,1	65kDa
3	3.9	50kDa
	(this band has now been shown to correspond to fragments from 2 or 3 proteins identified as bovine COMP and either bovine alpha-1 antiprotease inhibitor or Endopin-1)	
4	3.7	45kDa
5	3.4	40kDa
6	4.6	50kDa
7	6.3	67kDa
8	6.1	65kDa
9	6.3	60kDa
10	6.3	38kDa
11	6.0	30kDa
12	6.4	25kDa
13	6.8	22kDa
14	7.8	20kDa
15	8.2	18kDa
16	8.3	19kDa
17	8.6	20kDa
18	9.1	19kDa
19	5.3	12kDa
20	6.2	22kDa
21	7.2	21kDa

(i)   Cartilage oligomeric matrix protein [Fragment] - bovine

```
          10          20          30          40          50          60
          |           |           |           |           |           |
DGVLNEKDNC PLVRNPDQRN TDGDKWGDAC DNCRSQKNDD QKDTDKDGRG DACDDDIDGD

          70          80          90          100         110         120
          |           |           |           |           |           |
RIRNPVDNCP KVPNSDQKDT DGDGVGDACD NCPQKSNADQ RDVDHDFVGD ACDSDQDQDG

          130         140         150         160         170         180
          |           |           |           |           |           |
DGHQDSKDNC PTVPNSAQQD SDHDGQGDAC DDDDDNDGVP DSRDNCRLVP NPGQEDMDRD

          190         200         210         220         230         240
          |           |           |           |           |           |
GVGDACQGDF DADKVVDKID VCPENAEVTL TDFRAFQTVV LDPEGDAQID PNWVVLNQGM

          250         260         270         280         290         300
          |           |           |           |           |           |
EIVQTMNSDP GLCVGYTAFN GVDFEGPFHV NTATDDDYAG FIFGYHHSSS FYVVMWKQME

          310         320         330         340         350         360
          |           |           |           |           |           |
QTYWQANPFR AVAEPGIQLK AVKSSTGPGE QLRNALWHTG DTASQVRLLW KDPRNVGWKD

          370         380         390         400         410         420
          |           |           |           |           |           |
KTSYRWFLQH RPQVGYIRVR FYEGPELVAD SNVILDTTMR GGRLGVFCFS QENIIWANLR

          430
          |
YRCNDTIPED YEAQRLLQA
```

(ii) Odorant-binding protein - bovine

```
          10          20          30          40          50          60
          |           |           |           |           |           |
AQEEEAEQNL SELSGPWRTV YIGSTNPEKI QENGPFRTYF RELVFDDEKG TVDFYFSVKR

          70          80          90          100         110         120
          |           |           |           |           |           |
DGKWKNVHVK ATKQDDGTYV ADYEGQNVFK IVSLSRTHLV AHNINVDKHG QTTELTELFV

          130         140         150
          |           |           |
KLNVEDEDLE KFWKLTEDKG IDKKNVVNFL ENEDHPHPE
```

Figure 7

Shows the partial amino acid sequence of the bovine NC4 domain of type IX collagen alpha 1 chain sequence obtained from the ExPASy TrEMBL database on which the peptide sequences obtained from the MALD-MS peptide mass fingerprinting of gel spot 13 (Figure 4) has been superimposed as bolded type where they are identical.

```
        10           20           30           40           50           60
         |            |            |            |            |            |
PRFPVNSNSN GENELCPKVR IGQDDLPGFD LISQFQIDKA ASRRAIQRVV GSTALQVAYK

        70           80           90          100          110          120
         |            |            |            |            |            |
LGNNVDFRIP TRHLYPNGLP EEYSFLTTFR MTGSTLEKHW SIWQIQDSSG KEQVGVKING

       130          140          150          160          170          180
         |            |            |            |            |            |
QTKSVSFSYK GLDGSLQTAA FSNLPSLFDS QWHKIMIGVE RSSATLFVDC NRIESLPIKP
```

RGQIDVD

Figure 9:

Shows the amino acid sequence of the NC4 domain of the type IX collagen alpha 1 chain from 17 day old chick embryos sterna as reported by Vasios et al. (J Biological Chem. 263, 2324-2329, 1998) on which the amino acid sequences identified from the MALDI-MS analysis of the polypeptides separated by 2D electrophoresis have been superimposed indicating where the sequences are identical as bold type and underlined.

PRO-**ARG**-PHE-**PRO-VAL**-ASN-SER-ASN-SER-ASN / GLY-GLU-ASN-GLU-

LEU-CYS-PRO-LYS-VAL-**ARG** / **ILE-GLY-GLN-ASP-ASP-LEU-PRO-GLY-**

PHE-ASP / **LEU-ILE-SER-GLN-PHE-GLN-ILE**-ASP-LYS-ALA / ALA-SER-

ARG-ARG-ALA-ILE-**GLN-ARG-VAL-VAL** / **GLY-SER-THR-ALA-LEU**-GLN-

VAL-ALA-TRY-LYS / LEU-GLY-ASN-ASN-VAL-ASP-PHE-ARG / **THR**-ARG-

HIS-LEU-**TYR**-PRO-**ASN-GLY-LEU-PRO** / GLU-**GLU-TYR-SER-PHE-LEU-**

THR-THR-PHE-ARG / **MET-THR-GLY**-SER-**THR-LEU**-GLY-**LYS**-HIS-**TRP** /

SER-**ILE-TRP-GLN-ILE-GLN-ASP-SER-SER-GLY** / **LYS-GLU-GLN-VAL-**

GLY-VAL-LYS-ILE-**ASN-GL**Y / GLN-THR-**LYS-SER-VAL**-SER-**PHE-SER-**

TRY-**LYS** / **GLY**-LEU-**ASP-GLY-SER-LEU-GLN-THR-ALA**-ALA / **PHE**-SER-

ASN-**LEU-PRO**-SER-**LEU-PHE-ASP-SER** / GLN-TRP-HIS-LYS-ILE-MET-

ILE-GLY-**VAL-GLU** / ARG-SER-**SER**-ALA-**THR-LEU-PHE**-VAL-**ASP-CYS** /

ASN-ARG-ILE-**GLU**-SER-**LEU**-PRO-**ILE-LYS-PRO**

Figure 10:

Shows the human NC4 domain of the type IX collagen alpha 1 chain (sequences 24-268) obtained from the Swiss-Prot & TrEMBL data-bases (released 07-June-2004) on which the amino acid sequences identified from the MALDI-MS analysis of the polypeptides separated by 2D electrophoresis have been superimposed showing where the sequences are identical as bold type and underlined. Sequence 1-23 is the signal sequence for the human NC4 domain of the type IX collagen alpha 1 chain.

```
1 Met Lys Thr Cys Trp Lys Ile Pro Val Phe Phe Phe Val
Cys Ser 16 Phe Leu Glu Pro Trp Ala Ser Ala 23 Ala Val
Lys Arg Arg Pro Arg 31 Phe Pro Val Asn Ser Asn Ser Asn
Gly Gly Asn Glu Leu Cys Pro 46 Lys Ile Arg Ile Gly Gln
Asp Asp Leu Pro Gly Phe Asp Leu Ile 61 Ser Gln Phe Gln
Val Asp Lys Ala Ala Ser Arg Arg Ala Ile Gln 76 Arg Val
Val Gly Ser Ala Thr Leu Gln Val Ala Tyr Lys Leu Gly 91
Asn Asn Val Asp Phe Arg Ile Pro Thr Arg Asn Leu Tyr Pro
Ser 106 Gly Leu Pro Glu Glu Tyr Ser Phe Leu Thr Thr Phe
Arg Met Thr 121 Gly Ser Thr Leu Lys Lys Asn Trp Asn Ile
Trp Gln Ile Gln Asp 136 Ser Ser Gly Lys Glu Gln Val Gly
Ile Lys Ile Asn Gly Gln Thr 151 Gln Ser Val Val Phe Ser
Tyr Lys Gly Leu Asp Gly Ser Leu Gln 166 Thr Ala Ala Phe
Ser Asn Leu Ser Ser Leu Phe Asp Ser Gln Trp 181 His Lys
Ile Met Ile Gly Val Glu Arg Ser Ser Ala Thr Leu Phe 196
Val Asp Cys Asn Arg Ile Glu Ser Leu Pro Ile Lys Pro Arg
Gly 211 Pro Ile Asp Ile Asp Gly Phe Ala Val Leu Gly Lys
Leu Ala Asp 226 Asn Pro Gln Val Ser Val Pro Phe Glu Leu
Gln Trp Met Leu Ile 241 His Cys Asp Pro Leu Arg Pro Arg
Arg Glu Thr Cys His Glu Leu 256 Pro Ala Arg Ile Thr Pro
Ser Gln Thr Thr Asp Glu Arg 268
```

Figure 11

Protocols used to evaluate polypeptides (PPs) and drugs in the rat CIA model
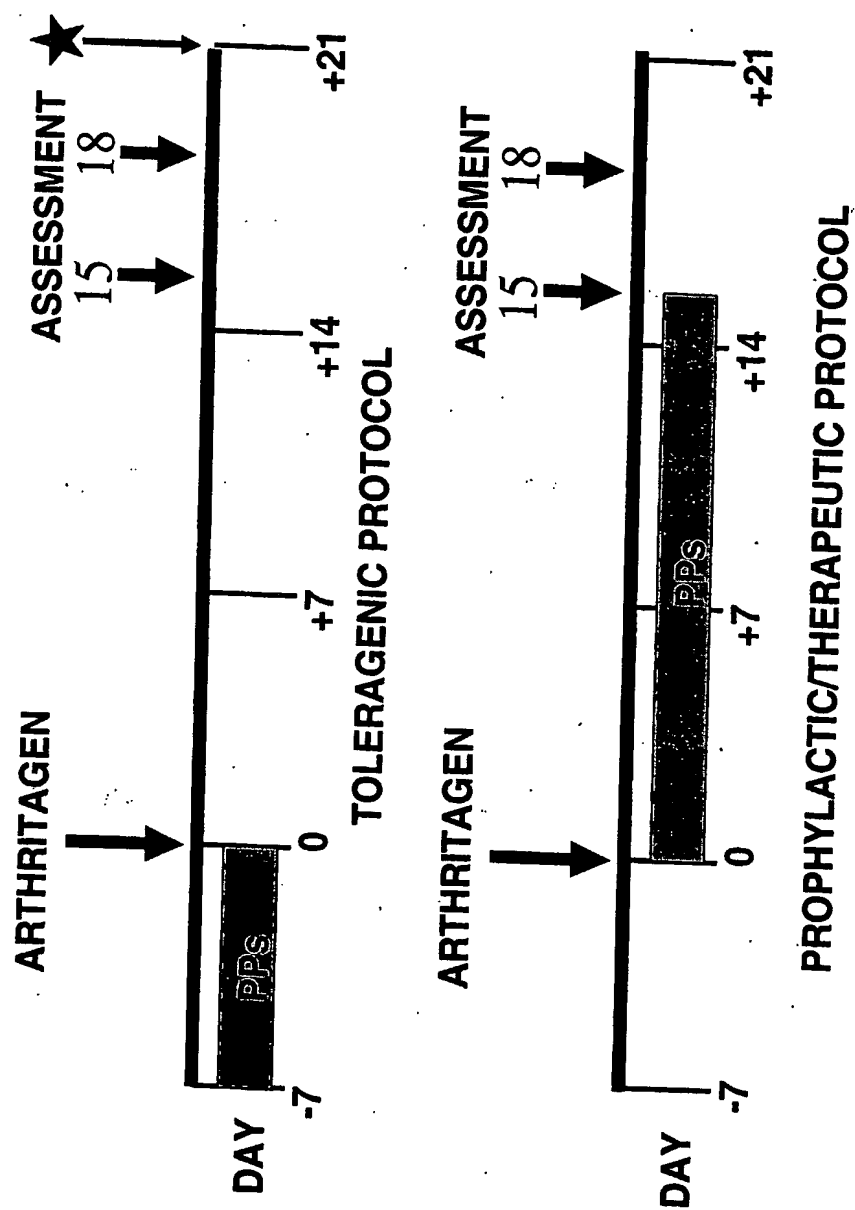
Figure 12

Results demonstrating anti-arthritic activity of the polypeptides INR-195 and INR-126 relative to ATM in the Rat CIA model using the Prophylactic/Therapeutic protocol(15 days treatment)

Rx n=4	Dose mg/kg	Mean arthritis scores			Signs of arthritis Day 15			Signs of arthritis Day 18		
		Day 13	Day 15	Day 18	R/paw swell (mm)	F/paw swell (mm)	Weight change (grams)	R/paw swell (mm)	F/paw swell (mm)	Weight change (grams)
None	—	0.7	1.5	1.8	0.7	2.1	+40	0.8	2.0	-04
INR-126	20 (oral)	0.2**	0.8*	1.3	0.4	1.3*	+47	0.7	2.0	+06
INR-126	200 (oral)	0.2**	0.5*	1.7	0.1	0.1**	+58	0.8	2.3	+04
INR-195	20 (oral)	0.2*	0.7*	1.8	0.2	0.1**	+45	0.7	2.0	+09
ATM	6.3 (SC)	0.8	2.1	2.1	0.7	2.5	+47	0.8	3.2	+04

ATM = Aurothiomalate, SC = subcutaneously, ** = $p < 0.005$ * = $p < 0.05$ relative to none

Figure 13

Results demonstrating the anti-arthritic activity of the polypeptides INR-195 and INR-126 in the Rat CIA model using the toleragenic protocol where preparations are given for 7 days before inducing arthritis

Rx n=4	Dose mg/kg	Mean arthritis scores			Signs of arthritis Day 15			Signs of arthritis Day 18		
		Day 13	Day 15	Day 18	R/paw swell (mm)	F/paw swell (mm)	Weight change (grams)	R/paw swell (mm)	F/paw swell (mm)	Weight change (grams)
None	—	0.7	1.3	1.5	0.8	2.8	+58	0.8	2.5	-01
INR-126	20 (oral)	0.1**	0.6*	0.5**	0.2*	1.4*	+50	0.06***	1.1*	+09
INR-195	20 (oral)	0.6	0.5*	1.5	0.1*	0.1*	+44	0.6	1,5	+05

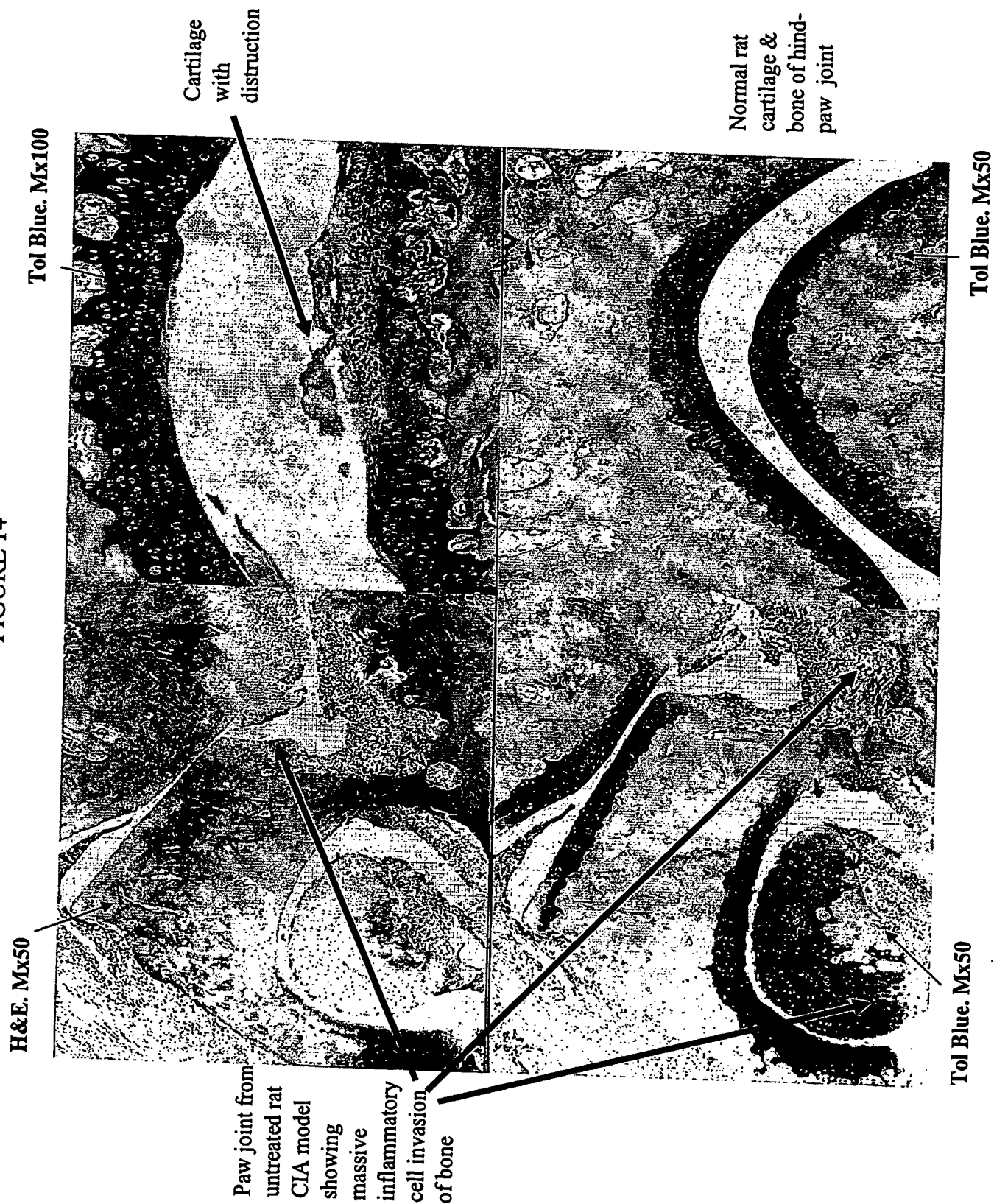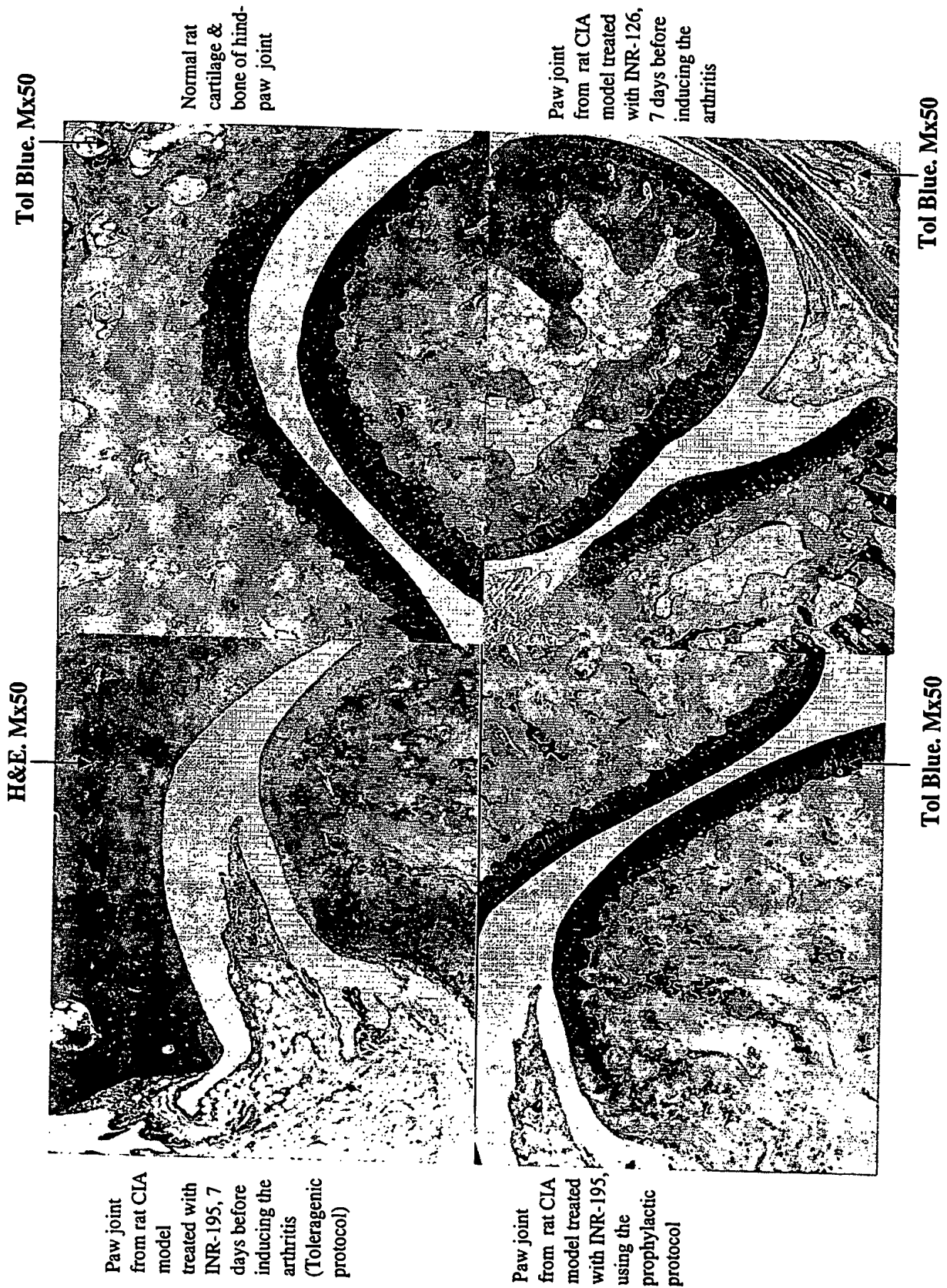** = $p < 0.005$ * = $p < 0.05$ relative to no treatment group

FIGURE 14

Tol Blue. Mx100

Cartilage with distruction

H&E. Mx50

Paw joint from untreated rat CIA model showing massive inflammatory cell invasion of bone

Normal rat cartilage & bone of hind-paw joint

Tol Blue. Mx50

Tol Blue. Mx50

FIGURE 15

Normal rat cartilage & bone of hind-paw joint

Tol Blue. Mx50

Paw joint from rat CIA model treated with INR-126, 7 days before inducing the arthritis

Tol Blue. Mx50

H&E. Mx50

Tol Blue. Mx50

Paw joint from rat CIA model treated with INR-195, 7 days before inducing the arthritis (Toleragenic protocol)

Paw joint from rat CIA model treated with INR-195, using the prophylactic protocol